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Dynamics and Determinants of HPV Infection: The Michigan HPV and Oropharyngeal Cancer (M-HOC) Study

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Abstract

Introduction: Human papillomavirus (HPV) is the primary cause of cervical and other anogenital cancers, and is also associated with head and neck cancers. Incidence of HPV-related oropharyngeal squamous cell cancers (OPSCCs) is increasing, and HPV-related OPSCCs have surpassed cervical cancer as the most common HPV-related cancer in the U.S. Given the multisite nature of HPV, there is strong interest in collecting data from both genital and oral sites, as well as associated data on social and sexual behaviors. The overarching goal of this study is to evaluate patterns of oral HPV infection incidence and clearance and their relationship to sexual history and long-term OPSCC risk.

Methods and Analysis: Participants are recruited from two populations: college students at a large public university and general population from the surrounding area. At the first study visit participants will complete a detailed sexual history, health, and behavior questionnaire. Follow-up visits will occur every 3–4 months over 3 years, when participants will complete an abbreviated questionnaire. All participants will provide a saliva sample at each visit, and eligible women may provide a cervical self-swab. Genetic material isolated from specimens will be tested for 15 high-risk and 3 low-risk HPV types. Statistical analyses will examine outcome variables including HPV prevalence, incidence, persistence, and clearance. Logistic regression models will be used to estimate odds ratios and 95% confidence intervals for associations between the outcomes of interest and demographic/behavioral variables collected in the questionnaires. The longitudinal HPV infection data and detailed sexual history data collected in the questionnaires will allow us to develop

individual-based network models of HPV transmission and will be used to parameterize multi-scale models of HPV-related OPSCC carcinogenesis.

Ethics and Dissemination: This study has been approved by the University of Michigan Institutional Review Board. All participants are consented in person by trained study staff.

Article Summary

Strengths and Limitations of this study

- Longitudinal design with 3–4 study visits per participant per year allows for analysis of incidence, persistence, and clearance of oral HPV.
- Collection of both oral and cervical samples allows for comparison of infections across sites.
- Low prevalence of high-risk oral HPV may limit our ability to connect HPV infection dynamics to demographic, sexual, and behavioral variables.

Introduction

Infectious disease agents account for approximately 20% of cancers worldwide [1]. The human papillomavirus (HPV) is the primary cause of cervical and most anogenital cancers, as well as an increasing fraction of head and neck cancers [2, 3]. Indeed, incidence of HPV-related oropharyngeal squamous cell cancers (OPSCCs) is increasing [4–7], and HPV-related OPSCCs have surpassed cervical cancer as the most common HPV-related cancer in the US [3]. At the University of Michigan Cancer Center, over 80% of OPSCC patients of have high-risk HPV detected in their cancers [8]. Although many HPV infections are benign, high-risk HPV infections can disrupt several key cancer regulation pathways, including particularly those involving tumor suppressors p53 and pRb [9, 10].

The underlying reasons behind the significant rise in HPV-related OPSCC rates in the U.S. are unclear. From a prevention perspective, addressing HPV-related OPSCC begins with an understanding of the epidemiology of the virus, both in the oral cavity and more broadly. Estimates from the National Health and Nutrition Examination survey (NHANES) estimate oral HPV prevalence in the U.S. to be about 11–12% for men and 3– 4 % for women [11–14]. While studies are increasingly treating HPV as a multisite disease, the mechanisms underlying the

relationship between oral, genital, and anal HPV infections are not understood, although autoinoculation is increasingly implicated [15, 16]. The HPV vaccine further complicates the epidemiological picture: HPV vaccination is expected to have an impact on the rates of oral HPV incidence, at least for those genotypes included in the vaccine [12, 17]. Countries with more aggressive vaccination campaigns, such as Australia, have already seen a reduction in HPV-related genital warts [18, 19]. It remains to be seen whether HPV vaccination will ultimately reduce the incidence and mortality of HPV-related head and neck cancers.

Many open questions remain regarding oral HPV transmission epidemiology, infection and persistence, the mechanisms of HPV-related HN carcinogenesis, and the connection between the ongoing oral HPV epidemic and the rising OPSCC incidence. The knowledge gaps surrounding the mechanisms and epidemiology of HPV in the oral cavity hamper our ability to develop more effective measures to prevent oral HPV infections and, in turn, reduce the burden of head and neck cancers. Prevalence studies can only draw associations between the burden and probable risk factors. Thus, longitudinal studies with frequent testing and assessment of recent sexual behavior are needed to understand HPV transmission, persistence, and clearance and to associate these events with certain behaviors. Here, we describe the methods, study design, and initial study population characteristics of a longitudinal study of oral HPV incidence, clearance, and related sexual behaviors, the Michigan HPV and Oropharyngeal Cancer (M-HOC) Study and its cervical HPV sub-study. Our research will contribute to a better understanding of the complex factors affecting patterns of HPV infection. This study will inform predictive and mechanistic models and develop effective prevention strategies for OPSCC and other HPV-related cancers.

Methods and Analysis

Objective

The overarching goal of this study is to evaluate patterns of oral HPV infection incidence and clearance and their relationship to sexual history and sexual behaviors. Participants are recruited to the study from two populations: college students at the University of Michigan (UM) (a large public university) and the general population of the surrounding area. As a secondary goal, we will also compare HPV infection patterns in the oral and cervical sites as part of a sub-study examining cervical HPV infections.

Subject enrollment and consent

Here, we describe the process for subject enrollment and consent (diagrammed in Figure 1).

Identification of Study Subjects. Participants are continuously recruited by one of three methods. 1) Advertisement: we place recruitment announcements in UM health clinics and area community sites (e.g. libraries, coffee shops, bus stops), as well as on UM clinical research volunteer's website (www.umclinicalstudies.org). In this method, individuals who are potentially interested in participating in the study contact the study coordinator to determine eligibility. 2) Screening clinics: we approach participants of the UM health system free throat cancer screening clinic to invite eligible participants to join the study. 3) Student residence halls: once per year, we set up information tables in or near UM residence halls and invite eligible students to join the student.

Eligibility Criteria. Volunteers are considered for enrollment if they

- Are age 18 or older,
- Do not have a history of head or neck cancer,
- Are willing to return for a follow up every 3–4 months over 3 years,
- And are able and willing to provide written informed consent.

To participate in the optional cervical sub-study, participants must additionally

- Have female genitals,
- Not be pregnant,
- And not be menstruating at the time of the study visit.

Consent. We document informed consent for all willing and eligible participants before enrollment in the study. Separate, additional consent is obtained prior to enrollment in the cervical sub-study. All study subjects are given the opportunity to consent to storage of residual specimens collected in this study for future research purposes. Specimens collected from subjects who do not consent to specimen banking will be destroyed after completion of the analyses of this study.

Enrollment targets. We plan to enroll up to 1,000 individuals to participate over the course the first 5 years of this study. This study has been in active recruitment since April 2015 and has thus far (as of November 2017) enrolled 341 individuals who have completed the baseline questionnaire. Of these, 220 have completed at least one follow-up visit.

Figure 1: Flow diagram for the study detailing recruitment methods, visit structure (baseline and followups), and the cervical sub-study.

Data collection

We administer surveys and collect biological specimens as described below.

Social and Sexual Behavior Survey. All study participants complete a comprehensive questionnaire at the baseline visit and an abbreviated questionnaire at all subsequent visits. The questionnaire consists of questions related to demographic information; alcohol, cigarette, cigar, tobacco, and other drug use; and sexual practices, sexual history, sexual healthcare, general health, and history of sexually transmitted infections (STIs). The baseline questionnaire collects a complete sexual history, with subsequent follow up visits providing more recent information and updates. The questions regarding sexual behaviors and history are designed to provide detailed information about the timing of different sexual partnerships and sexual practices in each sexual partnership and form a longitudinal, egocentric sexual network data set. Table 1 details the categories and numbers of questions given in both the baseline and follow-up surveys. The questionnaire is administered electronically via Qualtrics in a quiet, private room. Participants are asked to enter their unique study identification number at the beginning of the questionnaire. Participants are free to skip or otherwise not answer any question.

Table 1: Categories and numbers of questions asked in MHOC study baseline and follow-up questionnaire.

Question category	Number of questions	
	Baseline	Follow-up
Demographic	8	5
Oral health and cancer	5	5
STI testing and diagnosis	17	12
Sexual health and vaccinations	25	16
Sexual behavior	70	6
Sexual partners	22	18
Substance use	20	16

Oral Specimen Collection. Two mL of saliva and oral rinse specimen are collected at each visit (i.e. baseline and every 3-4 months for 3 years) using Oragene RE-100 kits [20]. Participants may use a sweetener or candy to stimulate saliva production if needed.

In the initial phase of the study, we tested several alternative saliva collection methods: Scope-brand mouthwash (administered as in NHANES [21]), and two commercially available kits developed for detection of DNA and RNA in saliva, the OM-505 (suitable for DNA, stable for weeks at room temperature) and RE-100 (designed to protect RNA, stable for 60 days at room temperature) [20, 22]. We were able to detect DNA from saliva in all three methods but were only able to reliably recover RNA from the RE-100 kit sample. We selected the RE-100 kits for use in the study so that we could isolate both DNA and RNA from a single sample.

Cervical swab collection. Participants fulfilling the eligibility criteria are given the option at each study visit to provide a self-collected cervical swab sample using a HerSwab [23] in addition to the oral specimen. Participants self-administer the swab by inserting the tip of the HerSwab into the vagina; turning the crank so that the brush is fully extended; removing the swab; turning the crank the opposite direction to retract the brush back into the tip; and placing the HerSwab into a provided resealable plastic bag. The participants are provided with the printed, graphical instructions designed by the manufacturer and collect their sample in a restroom. Staff facilitating the study are available to review the procedure and answer any participant questions.

Cervical swab acceptability survey. All study participants who provide a cervical sample complete a questionnaire after each cervical self-collection that asks about comfort, ease-of-use, and subjective assessment of the self-collection procedure [24]. The questionnaire is administered electronically via Qualtrics.

Follow-up visit schedule

Participants are followed for up to three years after their baseline visit, for a total of up to 12 study visits. The study prioritizes longitudinal follow-up over regular follow-up, but strives to achieve both. Therefore, if participants miss a follow-up visit, they are not excluded and instead continue the study when next available. This protocol facilitates research in the student population, as students are often gone for extended periods of time on scheduled breaks.

Sample processing and analysis

Sample processing and DNA extraction differs for oral and cervical samples, but DNA analysis is the same for both kinds of samples.

Oral sample processing. DNA isolation from saliva samples is performed by first splitting the sample in half, half for DNA and half for RNA. The saliva is heated to 50°C for 60 minutes, 1/25th volume of prepIT-L2P (DNA Genotek) is added. Sample is incubated for 10 minutes at 4°C and spun for 20 minutes at 4100 rpm. After the supernatant is added to equal volumes of 95% ethanol to precipitate the DNA. The DNA is pelleted, washed with 200 μL of 70% ethanol and dried. The DNA pellet is rehydrated with Tris-EDTA buffer and quantified with picogreen (Invitrogen P11496) or the QuBit (Invitrogen Q32850). RNA is isolated from samples that have tested positive for HPV DNA. RNA isolation from saliva samples is performed using the Oragene RNA isolation method according to the manufacturer's instruction which includes QIAgen's RNeasy Micro Kit (74004). In short, the samples are heated to 50°C for 60 minutes, 95°C for 15 minutes, 20 μL neutralization solution is added. Samples then are incubated on ice for 10 minutes followed by the RNeasy Kit protocol. The RNA is then are converted to cDNA for further testing. The cDNA synthesis is preformed using Invitrogen's Superscript III protocol and reagents (18080-044).

Cervical sample processing. After collection, the HerSwab is soaked and swished vigorously, for one minute each, in 20 mL of ThinPrep solution. The 20 mL solution is then split evenly into two 15 mL nuclease-free conical tubes—one for RNA processing and one for DNA—and the tube for RNA is centrifuged. The pellet for RNA use is resuspended in RNAlater for isolation and later study. DNA and RNA processing is the same as above. For RNA isolation, the cells in RNAlater are pelleted by centrifugation and RNA is isolated using the RNeasy as described above. Qiagen reagents are used for DNA isolation. The cells in the ThinPrep sample are pelleted, resuspended in cell lysis buffer, incubated with Proteinase k, treated with protein precipitation

buffer, vortexed vigorously, pelleted and placed on ice for 10 minutes. The supernatant is transferred to isopropanol in glycogen solution mixed, and the DNA pellet is washed twice with cold ethanol, re-hydrated in hydration solution and dissolved at 65˚C, and DNA is quantified with picogreen or QuBit.

DNA analysis. DNA and cDNA samples are assayed using a previously described, highly-sensitive method [8]. Briefly, multiplex competitive PCR amplification of the heterogenous E6 region of 15 discrete high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73) and 3 low-risk (6, 11, 90) HPV types is performed, followed by probe-specific single base extension. Extension products are loaded onto a matrix silicon chip array and separated by size using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectroscopy, allowing detection of any HPV types present in the sample.

Statistical analysis

Outcomes of interest include, but are not limited to, HPV prevalence, incidence, persistence, and clearance. Covariates of interest include, but are not limited to, sex, age, student status, HIV-status, sexual orientation, HPV vaccine status, circumcision status, number of vaginal, oral, and anal sexual partners, timing and nature of recent sexual activity, use of sexual protection, and substance use. Demographic and sexual behavior differences among subpopulations will be considered.

Logistic regression models will be used to estimate odds ratios and 95% confidence intervals for associations between the outcomes of interest and the demographic/behavioral variables. Variables that are significant in univariate analysis or are considered to be relevant based on a priori knowledge will be evaluated in multiple logistic regression models. Cox regression models will be used to investigate the effect of covariates on clearance. Analyses will be performed in SAS or R statistical softwares.

Modeling analysis

The longitudinal HPV infection data and detailed sexual partner and sexual history data collected in the questionnaires will allow us to develop individual-based network models of HPV transmission. While the data from the study are necessarily egocentric (i.e. we cannot link partners reported by participants to any specific individual in or out of the study to form a full

sexual network), they can nonetheless be used to directly parameterize simulation models of transmission. The models we develop can match the timing of partner switching, numbers of partners, and type of sexual contact, as well as a range of behavioral and demographic covariates as well as HPV infections and clearance times. This will allow us to generalize our statistical model findings to different populations and to examine counterfactual scenarios of alternative HPV vaccine coverage levels. Similar models have been developed for other studies, both for sexually transmitted infections and other infectious diseases [25–30]. This study is designed to specifically inform such modeling analyses.

Ethics

The MHOC study was approved by the University of Michigan Institutional Review Board on August 26, 2014 (IRB # HUM00090326). This study was deemed to not require additional safety monitoring beyond the monitoring of the institutional review board. All study staff interacting with participants and participant data complete the University of Michigan Program for Education and Evaluation in Responsible Research and Scholarship (PEERRS) prior to beginning the project [31]. Informed consent is obtained from each participant by the study coordinator and PEERRS certified research assistants.

Discussion

The knowledge gained from this study will contribute to the field of HPV research, and will be disseminated via peer reviewed publications and presentations at national and international conferences. This study will provide improved estimates of HPV clearance rates in the oral cavity, due to the short follow-up times, and will also allow us to examine how HPV clearance is affected by a range of factors, including coinfection with multiple HPV types. More broadly, this study will improve our understanding of the relationships between behavior patterns and HPV, helping to elucidate how sexual behaviors, numbers of partners, and other behaviors (e.g. alcohol and substance use) relate to HPV infection patterns. Additionally, this study will provide detailed data on HPV transmission in the college student population—a key population who are also seeing changing vaccination patterns as HPV vaccine coverage improves. This will allow us to further examine the impact of HPV vaccination on the incidence and prevalence of a range of vaccine types (both those covered by the vaccine and others). The study questionnaire and

longitudinal design also are also useful in developing sexual network models of transmission, which will allow us to examine alternative vaccination strategies and generalize to other populations.

Limitations and Strengths

Because oral HPV prevalence in the United States is relatively low (prevalence of high-risk oral HPV among adults 18–69 was 4.0% in 2011–2014: 6.8% in men and 1.2% in women [14]), there is a risk that number of HPV positive samples in the study will be low, limiting our abilities to draw inference between HPV infection and the demographic and behavioral variables of the participant.

Instead of identifying risk factors through associations in cross-sectional prevalence analyses, we will use a longitudinal approach with frequent oral and cervical HPV testing and updated detailed sexual behavior questionnaires. This will allow for understanding not only prevalence of HPV but also incidence, persistence, and clearance. Some studies have also adopted a longitudinal approach (e.g. [32–36]), but many have had long times between follow-up or have focused only on sex or only on genital infection sites. In the MHOC study, we use short follow-up times, with 3–4 visits per participant per year. We focus primarily on oral infection in both men and women but also collect cervical samples to assess multisite infections.

Author's contributions

The study was conceptualized and funding was obtained by MCE, TEC, and RM. Methodology and original protocols were developed by MCE, LPC, AFB, HMW, BMM, YKL, TBT, RLD, CMG, TEC, and RM. Project administration is managed by LPC and YKL. MCE, LPC, AFB, HMW, BMM, YKL, CG, TEC, and RM are responsible for supervision of study implementation, staff, and students.

The original draft was prepared by MCE with the assistance of LPC, TSS, and MLY. Review and editing was completed by MCE, LPC, AFB, HMW, BMM, YKL, TEC, and RM.

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Competing interests statement

All authors declare no conflicts of interest.

References

[1] Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens–Part B: biological agents. The Lancet Oncology. 2009;10(4):321–322. Available from: http://dx.doi.org/10.1016/S1470-2045(09)70096-8.

[2] Gillison ML, Alemany L, Snijders PJF, Chaturvedi A, Steinberg BM, Schwartz S, et al. Human papillomavirus and diseases of the upper airway: head and neck cancer and respiratory papillomatosis. Vaccine. 2012 nov;30 Suppl 5:F34–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23199965.

[3] Jemal A, Simard EP, Dorell C, Noone AM, Markowitz LE, Kohler B, et al. Annual Report to the Nation on the Status of Cancer,
1975-2009, Featuring the Burden and Trends in Human Papillomavirus (HPV)-Associated Cancers and HPV Vaccination Coverage Levels. Journal of the National Cancer Institute. 2013;105(3):175–201. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3565628{\&}tool=pmcentrez{\&}rendertype=abstract.

[4] Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. Journal of Clinical Oncology. 2008;26(4):612–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18235120.

[5] Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. Journal of Clinical Oncology. 2011;29(32):4294–301. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3221528{\&}tool=pmcentrez{\&}rendertype=abstract.

[6] Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. Journal of Clinical Oncology. 2013 dec;31(36):4550–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24248688.

[7] Chaturvedi AK, D'Souza G, Gillison ML, Katki HA. Burden of HPV-positive oropharynx cancers among ever and never smokers in the U.S. population. Oral Oncology. 2016;60:61–67. Available from: http://www.sciencedirect.com/science/article/pii/ S136883751630080X.

[8] Walline HM, Komarck C, McHugh JB, Byrd SA, Spector ME, Hauff SJ, et al. High-Risk Human Papillomavirus Detection in Oropharyngeal, Nasopharyngeal, and Oral Cavity Cancers. JAMA Otolaryngology–Head & Neck Surgery. 2013;139(12):1320.
Available from: http://archotol.jamanetwork.com/article.aspx?doi=10.1001/jamaoto.2013.5460.

[9] Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine.
2012 nov;30 Suppl 5:F55–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23199966.

[10] McLaughlin-Drubin ME, Munger K. Oncogenic activities of human papillomaviruses. Virus Research. 2009;143(2):195–208.¨

[11] Gillison ML, Broutian T, Pickard RKL, Tong Zy, Xiao W, Kahle L, et al. Prevalence of oral HPV infection in the United States, 2009-2010. Journal of the American Medical Association. 2012;307(7):693–703. Available from: http://www.ncbi.nlm.nih. gov/pubmed/22282321.

[12] Brouwer AF, Eisenberg MC, Carey TE, Meza R. Trends in HPV cervical and seroprevalence and associations between oral and genital infection and serum antibodies in NHANES 2003-2012. BMC Infectious Diseases. 2015 dec;15(1):575. Available from: http://www.biomedcentral.com/1471-2334/15/575.

[13] Sonawane K, Suk R, Chiao EY, Chhatwal J, Qiu P, Wilkin T, et al. Oral Human Papillomavirus Infection: Differences in Prevalence Between Sexes and Concordance With Genital Human Papillomavirus Infection, NHANES 2011 to 2014. Annals of Internal Medicine. 2017;Available from: http://annals.org/aim/article/2657698/
oral-human-papillomavirus-infection-differences-prevalence-between-sexes-concordance-genital.

[14] McQuillan G, Kruszon-moran D, Markowitz LE, Unger ER, Paulose-ram R. Prevalence of HPV in Adults Aged 18–69: United States,
2011–2014. NCHS Data Brief. 2017;280. Available from: https://www.cdc.gov/nchs/products/databriefs/db280.htm.

[15] Pamnani SJ, Nyitray AG, Abrahamsen M, Rollison DE, Villa LL, Lazcano-Ponce E, et al. Sequential Acquisition of Anal Human Papillomavirus (HPV) Infection Following Genital Infection among Men Who Have Sex with Women: The HPV Infection in Men (HIM) Study. Journal of Infectious Diseases. 2016;214(8):1180–1187.

[16] Ranjeva S, Baskerville EB, Dukic V, Villa L, Lazcano-Ponce E, Giuliano AR, et al. Recurring infection with ecologically distinct human papillomavirus (HPV) types explains high

prevalence and diversity. bioRxiv. 2017;Available from: https://www.biorxiv.org/content/early/2017/08/23/179341.

[17] Markowitz LE, Liu G, Hariri S, Steinau M, Dunne EF, Unger ER. Prevalence of HPV After Introduction of the Vaccination Program in the United States. Pediatrics. 2016;137(3):1–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26908697.

[18] Ali H, Donovan B, Wand H, Read TRH, Regan DG, Grulich aE, et al. Genital warts in young Australians five years into national human papillomavirus vaccination programme: national surveillance data. BMJ. 2013;346:2032–2032. Available from: http://www.bmj.com/cgi/doi/10.1136/bmj.f2032.

[19] Chow EPF, Read TRH, Wigan R, Donovan B, Chen MY, Bradshaw CS, et al. Ongoing decline in genital warts among young heterosexuals 7 years after the Australian human papillomavirus (HPV) vaccination programme. Sexually Transmitted Infections. 2015;91(3):214–219. Available from: http://sti.bmj.com/lookup/doi/10.1136/sextrans-2014-051813.

[20] DNA Genotek, Inc. Oragene-RNA (RE-100); 2017. Available from: http://www.dnagenotek.com/US/products/RE100.html.

[21] Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey (NHANES): HPV rinse; 2009. Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2009-2010/manuals/HPV.pdf.

[22] DNA Genotek, Inc. OMNIgene-ORAL (OM-505); 2017. Available from: http://www.dnagenotek.com/US/products/OM505.html.

[23] Eve Medical. Eve Medical products: HerSwab; 2017. Available from: http://www.eve-medical.com/products.

[24] Gottschlich A, Rivera-Andrade A, Grajeda E, Alvarez C, Montano CM, Meza R. Acceptability of Human Papillomavirus SelfSampling for Cervical Cancer Screening in an Indigenous Community in Guatemala. Journal of Global Oncology. 2017;3(5):444– 454. Available from: https://doi.org/10.1200/JGO.2016.005629.

[25] Petrie JG, Eisenberg MC, Ng S, Malosh RE, Han Lee K, Ohmit SE, et al. Application of an Individual-Based Transmission Hazard Model for Estimation of Influenza Vaccine Effectiveness in a Household Cohort. American journal of epidemiology;.

[26] Aiello AE, Simanek AM, Eisenberg MC, Walsh AR, Davis B, Volz E, et al. Design and methods of a social network isolation study for reducing respiratory infection transmission: The eX-FLU cluster randomized trial. Epidemics. 2016;15:38–55.

[27] Fan K, Eisenberg M, Walsh A, Aiello A, Heller K. Hierarchical graph-coupled hmms for heterogeneous personalized health data. In: Proceedings of the 21th ACM SIGKDD International Conference on Knowledge Discovery and Data Mining. ACM; 2015. p. 239–248.

[28] Kaplan EH, Heimer R. HIV prevalence among intravenous drug users: model-based estimates from New Haven's legal needle exchange. JAIDS Journal of Acquired Immune Deficiency Syndromes. 1992;5(2):163–169.

[29] Sloot PM, Ivanov SV, Boukhanovsky AV, van de Vijver DA, Boucher CA. Stochastic simulation of HIV population dynamics through complex network modelling. International Journal of Computer Mathematics. 2008;85(8):1175–1187.

[30] Cohen T, Colijn C, Finklea B, Murray M. Exogenous re-infection and the dynamics of tuberculosis epidemics: local effects in a network model of transmission. Journal of The Royal Society Interface. 2007;4(14):523–531.

[31] University of Michigan. Responsible Conduct of Research (RCR) Training; 2017. Available from: http://research-compliance. umich.edu/responsible-conduct-research-rcr-training.

[32] Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. The New England Journal of Medicine. 1998;338(7):423–8. Available from: http://www.nejm.org/doi/full/10.1056/NEJM199802123380703http://www.ncbi.nlm.nih.gov/pubmed/9459645.

[33] Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, D´esy M, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. Journal of Infectious Diseases. 1999 nov;180(5):1415–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10515798.

[34] Molano M, Van den Brule A, Plummer M, Weiderpass E, Posso H, Arslan A, et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. American Journal of Epidemiology. 2003

sep;158(5):486–94. Available from: http://aje.oupjournals.org/cgi/doi/10.1093/aje/kwg171http://www.ncbi.nlm.nih.gov/pubmed/12936904.

[35] Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Thompson PJ, et al. Sequential acquisition of human papillomavirus (HPV) infection of the anus and cervix: the Hawaii HPV Cohort Study. The Journal of Infectious Diseases. 2010;201(9):1331–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2851490{\&}tool=pmcentrez{\&}rendertype=abstract.

[36] Giuliano AR, Lee JH, Fulp W, Villa LL, Lazcano E, Papenfuss MR, et al. Incidence and clearance of genital human papillomavirus infection in men (HIM): a cohort study. Lancet. 2011;377(9769):932–40. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3231998{\&}tool=pmcentrez{\&}rendertype=abstract.

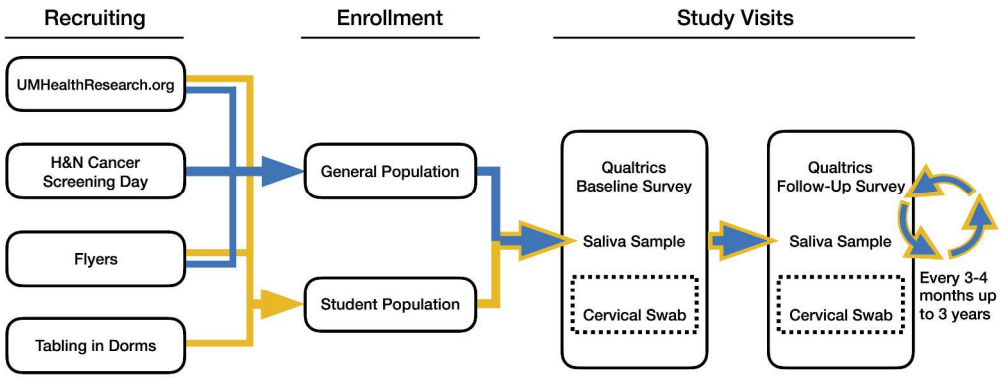

Figure 1: Flow diagram for the study detailing recruitment methods, visit structure (baseline and followups), and the cervical sub-study.

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Dynamics and Determinants of HPV Infection from the Michigan HPV and Oropharyngeal Cancer (M-HOC) Study

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Dynamics and Determinants of HPV Infection from the Michigan HPV and Oropharyngeal Cancer (M-HOC) Study

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Abstract

Introduction: Human papillomavirus (HPV) is the primary cause of cervical and other anogenital cancers, and is also associated with head and neck cancers. Incidence of HPV-related oropharyngeal squamous cell cancers (OPSCCs) is increasing, and HPV-related OPSCCs have surpassed cervical cancer as the most common HPV-related cancer in the U.S. Given the multisite nature of HPV, there is strong interest in collecting data from both genital and oral sites, as well as

associated data on social and sexual behaviors. The overarching goal of this study is to evaluate patterns of oral HPV infection incidence, clearance, and persistence and their relationship to sexual behavior history.

Methods and Analysis: Participants are recruited from two populations: college students at a large public university and general population from the surrounding area. At the first study visit participants will complete a detailed sexual history, health, and behavior questionnaire. Follow-up visits will occur every 3–4 months over 3 years, when participants will complete an abbreviated questionnaire. All participants will provide a saliva sample at each visit, and eligible participants may provide a cervicovaginal self-swab. Genetic material isolated from specimens will be tested for 15 high-risk and 3 low-risk HPV types. Statistical analyses will examine outcome variables including HPV prevalence, incidence, persistence, and clearance. Logistic regression models will be used to estimate odds ratios and 95% confidence intervals for associations between the outcomes of interest and demographic/behavioral variables collected in the questionnaires. The longitudinal HPV infection data and detailed sexual history data collected in the questionnaires will allow us to develop individual-based network models of HPV transmission and will be used to parameterize multi-scale models of HPV-related OPSCC carcinogenesis.

Ethics and Dissemination: This study has been approved by the University of Michigan Institutional Review Board. All participants are consented in person by trained study staff.

Article Summary

Strengths and Limitations of this study

- Longitudinal design with 3–4 study visits per participant per year allows for analysis of incidence, persistence, and clearance of oral HPV.

- Collection of both oral and cervicovaginal samples allows for comparison of infections across sites.
- Low prevalence of high-risk oral HPV may limit our ability to connect HPV infection dynamics to demographic, sexual, and behavioral variables.

Introduction

Infectious disease agents account for approximately 20% of cancers worldwide [1]. The human papillomavirus (HPV) is the primary cause of cervical and most anogenital cancers, as well as an increasing fraction of head and neck cancers [2, 3]. Indeed, incidence of HPV-related oropharyngeal squamous cell cancers (OPSCCs) is increasing [4–7], and HPV-related OPSCCs have surpassed cervical cancer as the most common HPV-related cancer in the US [3]. At the University of Michigan Cancer Center, over 80% of OPSCC patients of have high-risk HPV detected in their cancers [8]. Although many HPV infections are benign, high-risk HPV infections can disrupt several key cancer regulation pathways, including particularly those involving tumor suppressors p53 and pRb [9, 10].

The underlying reasons behind the significant rise in HPV-related OPSCC rates in the U.S. are unclear. From a prevention perspective, addressing HPV-related OPSCC begins with an understanding of the epidemiology of the virus, both in the oral cavity and more broadly. Estimates from the National Health and Nutrition Examination survey (NHANES) suggest oral HPV prevalence in the U.S. is about 11–12% in men and 3–4 % in women [11–14]. However, while oral HPV infections may clear relatively quickly (the HPV in Men trial estimated a mean clearance time of approximately 7 months [15]), persistent infections may lead to cancer after several decades (although there are no precise estimates of time-to-cancer for OPSCCs, cervical lesions are estimated to progress to cancer in 10–30 years [16]).

While studies are increasingly treating HPV as a multisite disease, the mechanisms underlying the relationship between oral, genital, and anal HPV infections are not understood, although autoinoculation is increasingly implicated [17, 18]. The HPV vaccine further complicates the epidemiological picture: HPV vaccination is expected to have an impact on the rates of oral HPV incidence, at least for those genotypes included in the vaccine [12, 19]. Countries with more aggressive vaccination campaigns, such as Australia, have already seen a reduction in HPV-related genital warts [20, 21]. It remains to be seen whether HPV vaccination will ultimately reduce the incidence and mortality of HPV-related head and neck cancers.

Many open questions remain regarding oral HPV transmission, epidemiology, infection and persistence, the mechanisms of HPV-related HN carcinogenesis, and the connection between the ongoing oral HPV epidemic and the rising OPSCC incidence. The knowledge gaps surrounding the mechanisms and epidemiology of HPV in the oral cavity hamper our ability to develop more effective measures to prevent oral HPV infections and, in turn, reduce the burden of head and neck cancers. Prevalence studies can only draw associations between the burden and probable risk factors. Thus, longitudinal studies with frequent testing and assessment of recent sexual behavior are needed to understand HPV transmission, persistence, and clearance and to associate these events with certain behaviors. Here, we describe the methods, study design, and initial study population characteristics of a longitudinal study of oral HPV incidence, clearance, and related sexual behaviors; this study and its cervicovaginal HPV sub-study are the epidemiological arms of the Michigan HPV and Oropharyngeal Cancer (M-HOC) Study. Our research will contribute to a better understanding of the complex factors affecting patterns of HPV infection. This study will inform predictive and mechanistic models and develop effective prevention strategies for OPSCC and other HPV-related cancers.

Methods and Analysis

Objective

The overarching goal of this study is to evaluate patterns of oral HPV infection incidence and clearance and their relationship to sexual history and sexual behaviors. Participants are recruited to the study from two populations: college students at the University of Michigan (UM) (a large public university) and the general population of the surrounding area. As a secondary goal, we will also compare HPV infection patterns in the oral and cervicovaginal sites as part of a sub-study examining cervicovaginal HPV infection.

Subject enrollment and consent

Here, we describe the process for subject enrollment and consent (diagrammed in Figure 1).

Identification of Study Subjects. Participants are continuously recruited by one of three methods. 1) Advertisement: we place recruitment announcements in UM health clinics and area community sites (e.g. libraries, coffee shops, bus stops), as well as on UM clinical research volunteer's website (www.umclinicalstudies.org). In this method, individuals who are potentially interested in participating in the study contact the study coordinator to determine eligibility. 2) Screening clinics: we approach participants of the UM health system free throat cancer screening clinic to invite eligible participants to join the study. 3) Student residence halls: once per year, we set up information tables in or near UM residence halls and invite eligible students to join the student.

Eligibility Criteria. Volunteers are considered for enrollment if they

- Are age 18 or older,

- Do not have a history of head or neck cancer,
- Are willing to return for a follow up every 3–4 months over 3 years,
- And are able and willing to provide written informed consent.

To participate in the optional cervicovaginal sub-study, participants must additionally

- Have a vagina,
- Not be pregnant,
- And not be menstruating at the time of the study visit.

Consent. We document informed consent for all willing and eligible participants before enrollment in the study. Separate, additional consent is obtained prior to enrollment in the cervicovaginal sub-study. All study subjects are given the opportunity to consent to storage of residual specimens collected in this study for future research purposes. Specimens collected from subjects who do not consent to specimen banking will be destroyed after completion of the analyses of this study.

Benefit to participants. Since we are not using a clinical test, our IRB approval does not allow returning individual HPV test results to participants, but population-level results will be disseminated through newsletters after peer-reviewed publication. Staff are knowledgeable and available to discuss vaccination and screening with interested participants. Because of the nature of the sexual and behavioral questionnaire, we also have pamphlets for the UM Sexual Assault Prevention and Awareness Center available to participants.

Enrollment and statistical power. Phase I of this study recruited between April 2015 and December 2017, enrolling 395 participants. At this sample size and level of significance 0.05, we will detect the difference at baseline between two equally sized populations with HPV

prevalence 10% and 20% with 80% power. Assuming each participant completes 10 visits, we will detect the difference between 10% and 13% ever HPV positive with more than 80% power. At the time of submission, 321 participants had completed at least one follow-up visit, and 1,693 baseline and follow-up visits had been completed. Follow-up visits are ongoing. Pending funding, phase II is anticipated to recruit a similar number of participants (potentially with additional study locations as well).

Figure 1: Flow diagram for the study detailing recruitment methods, visit structure (baseline and followups), and the cervicovaginal sub-study.

Data collection

We administer surveys and collect biological specimens as described below.

Social and Sexual Behavior Survey. All study participants complete a comprehensive questionnaire at the baseline visit and an abbreviated questionnaire at all subsequent visits. The questionnaire consists of questions related to demographic information; behavior, including alcohol, cigarette, cigar, tobacco, and other drug use; and sexual practices, sexual history, sexual healthcare (including HPV vaccination status), general health, and history of sexually transmitted infections (STIs). The baseline questionnaire collects a complete sexual history, with subsequent follow up visits providing more recent information and updates. The questions regarding sexual behaviors and history are designed to provide detailed information about the timing of different sexual partnerships and sexual practices in each sexual partnership and form a longitudinal, egocentric sexual network data set. Table 1 details the categories and numbers

of questions given in both the baseline and follow-up surveys. The questionnaire is administered electronically via Qualtrics in a quiet, private room. Participants are asked to enter their unique study identification number at the beginning of the questionnaire. Participants are free to skip or otherwise not answer any question.

Table 1: Categories and numbers of questions asked in MHOC study baseline and follow-up questionnaire.

	Number of questions	
Question category	Baseline	Follow-up
Demographic	8	5
Oral health and cancer	5	5
STI testing and diagnosis	17	12
Sexual health and vaccinations	25	16
Sexual behavior	70	6
Sexual partners	22	18
Substance use	20	16

Oral Specimen Collection. Two mL of saliva and oral rinse specimen are collected at each visit (i.e. baseline and every 3-4 months for 3 years) using Oragene RE-100 kits [22]. Participants may use a sweetener or candy to stimulate saliva production if needed.

In the initial phase of the study, we tested several alternative saliva collection methods: Scope-brand mouthwash (administered as in NHANES [23]), and two commercially available kits developed for detection of DNA and RNA in saliva, the OM-505 (suitable for DNA, stable for weeks at room temperature) and RE-100 (designed to protect RNA, stable for 60 days at room temperature) [22, 24]. We were able to detect DNA from saliva in all three methods but were only able to reliably recover RNA from the RE-100 kit sample. We selected the RE-100 kits for use in the study so that we could isolate both DNA and RNA from a single sample.

Cervicovaginal swab collection. Participants fulfilling the eligibility criteria are given the option at each study visit to provide a self-collected cervicovaginal swab sample using a HerSwab [25] in

addition to the oral specimen. The HerSwab is a vaginal swab designed to sample near the cervix. Participants self-administer the swab by inserting the tip of the HerSwab into the vagina; turning the crank so that the brush is fully extended; removing the swab; turning the crank the opposite direction to retract the brush back into the tip; and placing the HerSwab into a provided resealable plastic bag. The participants are provided with the printed, graphical instructions designed by the manufacturer and collect their sample in a restroom. Staff facilitating the study are available to review the procedure and answer any participant questions.

Cervicovaginal swab acceptability survey. All study participants who provide a cervicovaginal sample complete a questionnaire after each cervicovaginal self-collection that asks about comfort, ease-of-use, and subjective assessment of the self-collection procedure [26]. The questionnaire is administered electronically via Qualtrics.

Follow-up visit schedule

Participants are followed for up to three years after their baseline visit, for a total of up to 12 study visits. The study prioritizes longitudinal follow-up over regular follow-up, but strives to achieve both. Therefore, if participants miss a follow-up visit, they are not excluded and instead continue the study when next available. This protocol facilitates research in the student population, as students are often gone for extended periods of time on scheduled breaks.

Sample processing and analysis

Sample processing and DNA extraction differs for oral and cervicovaginal samples, but DNA analysis is the same for both kinds of samples.

Oral sample processing. DNA isolation from saliva samples is performed by first splitting the sample in half, half for DNA and half for RNA. The saliva is heated to 50˚C for 60 minutes, 1/25th

volume of prepIT-L2P (DNA Genotek) is added. Sample is incubated for 10 minutes at 4°C and spun for 20 minutes at 4100 rpm. After the supernatant is added to equal volumes of 95% ethanol to precipitate the DNA. The DNA is pelleted, washed with 200 μL of 70% ethanol and dried. The DNA pellet is rehydrated with Tris-EDTA buffer and quantified with picogreen (Invitrogen P11496) or the QuBit (Invitrogen Q32850). RNA is isolated from samples that have tested positive for HPV DNA. RNA isolation from saliva samples is performed using the Oragene RNA isolation method according to the manufacturer's instruction which includes QIAgen's RNeasy Micro Kit (74004). In short, the samples are heated to 50°C for 60 minutes, 95°C for 15 minutes, 20 μL neutralization solution is added. Samples then are incubated on ice for 10 minutes followed by the RNeasy Kit protocol. The RNA is then converted to cDNA for further testing. The cDNA synthesis is preformed using Invitrogen's Superscript III protocol and reagents (18080-044).

Cervicovaginal sample processing. After collection, the HerSwab is soaked for one minute in 20 mL of ThinPrep solution and subsequently swished vigorously for one minute. The 20 mL solution is then split evenly into two 15 mL nuclease-free conical tubes—one for RNA processing and one for DNA—and the tube for RNA is centrifuged. The pellet for RNA use is resuspended in RNAlater for isolation and later study. DNA and RNA processing is the same as above. For RNA isolation, the cells in RNAlater are pelleted by centrifugation and RNA is isolated using the RNeasy as described above. Qiagen reagents are used for DNA isolation. The cells in the ThinPrep sample are pelleted, resuspended in cell lysis buffer, incubated with Proteinase k, treated with protein precipitation buffer, vortexed vigorously, pelleted and placed on ice for 10 minutes. The supernatant is transferred to isopropanol in glycogen solution mixed, and the DNA pellet is washed twice with cold ethanol, re-hydrated in hydration solution and dissolved at 65°C, and DNA is quantified with picogreen or QuBit.

DNA analysis. DNA and cDNA samples are assayed using a previously described, highly-sensitive method [8]. Briefly, multiplex competitive PCR amplification of the heterogeneous E6 region of 15 discrete high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73) and 3 low-risk (6, 11, 90) HPV types is performed, followed by probe-specific single base extension. Extension products are loaded onto a matrix silicon chip array and separated by size using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectroscopy, allowing detection of any HPV types present in the sample.

Statistical analysis

Outcomes of interest include, but are not limited to, HPV prevalence (detection of HPV, or detection of a specific HPV genotype), incidence (detection of HPV in a previously uninfected person, or detection of a specific HPV genotype in a person who previously tested negative for that genotype), persistence (detection of HPV at subsequent study visits, or detection of specific genotypes at subsequent study visits), and clearance (non-detection of HPV in a previously infected person, or non-detection of a specific HPV genotype in a person previously infected by that genotype). Other patterns of HPV detection, such as patterns of intermittent detection of the same genotype or detection of an HPV genotype at the oral site after previous detection at the genital site (or vice versa), will be considered. We will adjust for the time between visits as appropriate. Presence of RNA in addition to DNA will be used to distinguish between active and latent infections.

Covariates of interest include, but are not limited to, sex, age, student status, HIV-status, sexual orientation, HPV vaccine status, circumcision status, number of vaginal, oral, and anal sexual partners, timing and nature of recent sexual activity, use of sexual protection, and substance use. Demographic and sexual behavior differences among subpopulations will be considered.

Logistic regression models will be used to estimate odds ratios and 95% confidence intervals for associations between the outcomes of interest and the demographic/behavioral variables. Variables that are significant in univariate analysis or are considered to be relevant based on a priori knowledge will be evaluated in multiple logistic regression models. Cox regression models will be used to investigate the effect of covariates on clearance. Analyses will be performed in SAS or R statistical software.

Modeling analysis

The longitudinal HPV infection data and detailed sexual partner and sexual history data collected in the questionnaires will allow us to develop individual-based network models of HPV transmission. While the data from the study are necessarily egocentric (i.e. we cannot link partners reported by participants to any specific individual in or out of the study to form a full sexual network), they can nonetheless be used to directly parameterize simulation models of transmission. The models we develop can match the timing of partner switching, numbers of partners, and type of sexual contact, as well as a range of behavioral and demographic covariates as well as HPV infections and clearance times. We will draw from the measured distributions and correlation patterns of these variables in our population to generate simulated populations with sexual behavior patterns similar to those measured in our study (e.g. using approaches based on configuration model methods [27] to connect our simulated sexual network based on partner history data). This will allow us to generalize our statistical model findings to different populations and to examine counterfactual scenarios of alternative HPV vaccine coverage levels. Similar models have been developed for other studies, both for sexually transmitted infections and other infectious diseases [28–33]. This study is designed to specifically inform such modeling analyses.

Patient and Public Involvement

Patients and the public were not involved in the development of this study. Study results will be disseminated to the public through peer-reviewed publications as described in the ethics and dissemination section. Additionally, the newsletter described below will be made accessible to the public through the study website.

Ethics and dissemination

The MHOC study was approved by the University of Michigan Institutional Review Board on August 26, 2014 (IRB # HUM00090326). This study was deemed to not require additional safety monitoring beyond the monitoring of the institutional review board. All study staff interacting with participants and participant data complete the University of Michigan Program for Education and Evaluation in Responsible Research and Scholarship (PEERRS) prior to beginning the project [31]. Informed consent is obtained from each participant by the study coordinator and PEERRS certified research assistants. Study results will be disseminated through a series of peer-reviewed publications. Study participants will receive a study results newsletter written for a general audience.

Discussion

The knowledge gained from this study will contribute to the field of HPV research, and will be disseminated via peer reviewed publications and presentations at national and international conferences. This study will provide improved estimates of HPV clearance rates in the oral cavity, due to the short follow-up times, and will also allow us to examine how HPV clearance is

affected by a range of factors, including coinfection with multiple HPV types. More broadly, this study will improve our understanding of the relationships between behavior patterns and HPV, helping to elucidate how sexual behaviors, numbers of partners, and other behaviors (e.g. alcohol and substance use) relate to HPV infection patterns. Additionally, this study will provide detailed data on HPV transmission in the college student population—a key population who are also seeing changing vaccination patterns as HPV vaccine coverage improves. This will allow us to further examine the impact of HPV vaccination on the incidence and prevalence of a range of vaccine types (both those covered by the vaccine and others). The study questionnaire and longitudinal design also are also useful in developing sexual network models of transmission, which will allow us to examine alternative vaccination strategies and generalize to other populations.

Limitations and Strengths

Because oral HPV prevalence in the United States is relatively low (prevalence of high-risk oral HPV among adults 18–69 was 4.0% in 2011–2014: 6.8% in men and 1.2% in women [14]), there is a risk that number of HPV positive samples in the study will be low, limiting our abilities to draw inference between HPV infection and the demographic and behavioral variables of the participant. The quality of our saliva and oral rinse specimens may depend on the saliva production and swishing efficacy of each participant, although this is mitigated by the sensitivity of the PCR analysis. Finally, we only test for 18 genotypes, which, although we cover all high-risk types, may not give as complete a picture of patterns of mucosal HPV infection.

Instead of identifying risk factors through associations in cross-sectional prevalence analyses, we will use a longitudinal approach with frequent oral and cervicovaginal HPV testing and updated detailed sexual behavior questionnaires. This will allow for understanding not only prevalence of HPV but also incidence, persistence, and clearance. Some studies have also

adopted a longitudinal approach (e.g. [34–38]), but many have had long times between follow-up or have focused only on sex or only on genital infection sites. In the MHOC study, we use short follow-up times, with 3–4 visits per participant per year. We focus primarily on oral infection in both men and women but also collect cervicovaginal samples to assess multisite infections.

Author's contributions

The study was conceptualized and funding was obtained by MCE, TEC, and RM. Methodology and original protocols were developed by MCE, LPC, AFB, HMW, BMM, YKL, TBT, RLD, CMG, TEC, and RM. Project administration is managed by LPC and YKL. MCE, LPC, AFB, HMW, BMM, YKL, CG, TEC, and RM are responsible for supervision of study implementation, staff, and students.

The original draft was prepared by MCE with the assistance of LPC, TSS, and MLY. Review and editing was completed by MCE, LPC, AFB, HMW, BMM, YKL, TEC, and RM.

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Competing interests statement

All authors declare no conflicts of interest.

References

[1] Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens–Part B: biological agents. The Lancet Oncology. 2009;10(4):321–322.

[2] Gillison ML, Alemany L, Snijders PJF, Chaturvedi A, Steinberg BM, Schwartz S, et al. Human papillomavirus and diseases of the upper airway: head and neck cancer and respiratory papillomatosis. Vaccine. 2012 nov;30 Suppl 5:F34–54.

[3] Jemal A, Simard EP, Dorell C, Noone AM, Markowitz LE, Kohler B, et al. Annual Report to the Nation on the Status of Cancer,

1975-2009, Featuring the Burden and Trends in Human Papillomavirus (HPV)-Associated Cancers and HPV Vaccination Coverage Levels. Journal of the National Cancer Institute. 2013;105(3):175–201.

[4] Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. Journal of Clinical Oncology. 2008;26(4):612–9.

[5] Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. Journal of Clinical Oncology. 2011;29(32):4294–301.

[6] Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. Journal of Clinical Oncology. 2013 dec;31(36):4550–9.

[7] Chaturvedi AK, D'Souza G, Gillison ML, Katki HA. Burden of HPV-positive oropharynx cancers among ever and never smokers in the U.S. population. Oral Oncology. 2016;60:61–67.

[8] Walline HM, Komarck C, McHugh JB, Byrd SA, Spector ME, Hauff SJ, et al. High-Risk Human Papillomavirus Detection in Oropharyngeal, Nasopharyngeal, and Oral Cavity Cancers. JAMA Otolaryngology–Head & Neck Surgery. 2013;139(12):1320.

[9] Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30 Suppl 5:F55–70

[10] McLaughlin-Drubin ME, Munger K. Oncogenic activities of human papillomaviruses. Virus Research. 2009;143(2):195–208.¨

[11] Gillison ML, Broutian T, Pickard RKL, Tong Zy, Xiao W, Kahle L, et al. Prevalence of oral HPV infection in the United States, 2009-2010. Journal of the American Medical Association. 2012;307(7):693–703.

[12] Brouwer AF, Eisenberg MC, Carey TE, Meza R. Trends in HPV cervical and seroprevalence and associations between oral and genital infection and serum antibodies in NHANES 2003-2012. BMC Infectious Diseases. 2015;15(1):575.

[13] Sonawane K, Suk R, Chiao EY, Chhatwal J, Qiu P, Wilkin T, et al. Oral Human Papillomavirus Infection: Differences in Prevalence Between Sexes and Concordance With Genital Human Papillomavirus Infection, NHANES 2011 to 2014. Annals of Internal Medicine. 2017; 167(10):714-724.

[14] McQuillan G, Kruszon-moran D, Markowitz LE, Unger ER, Paulose-ram R. Prevalence of HPV in Adults Aged 18–69: United States, 2011–2014. NCHS Data Brief. 2017;280.

[15] Kreimer AR, Campbell, CMP, Lin H, Fulp W, Papenfuss MR, Abrahamsen M, Hildesheim A, Villa LL, Salmerón JJ, Lazcano-Ponce E, Giuliano AR. Incidence and clearance of oral human papillomavirus infection in men: the HIM cohort study. The Lancet. 2013;382(9895):877–887.

[16] McCredie M, Sharples KJ, Paul C, Baranyai J, Medley G, Jones RW, Skegg DCG. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. The Lancet Oncology. 2008;9(5):425–434.

[17] Pamnani SJ, Nyitray AG, Abrahamsen M, Rollison DE, Villa LL, Lazcano-Ponce E, et al. Sequential Acquisition of Anal Human Papillomavirus (HPV) Infection Following Genital Infection among Men Who Have Sex with Women: The HPV Infection in Men (HIM) Study. Journal of Infectious Diseases. 2016;214(8):1180–1187.

[18] Ranjeva S, Baskerville EB, Dukic V, Villa L, Lazcano-Ponce E, Giuliano AR, et al. Recurring infection with ecologically distinct human papillomavirus (HPV) types explains high prevalence and diversity. bioRxiv. 2017

[19] Markowitz LE, Liu G, Hariri S, Steinau M, Dunne EF, Unger ER. Prevalence of HPV After Introduction of the Vaccination Programin the United States. Pediatrics. 2016;137(3):1–9.

[20] Ali H, Donovan B, Wand H, Read TRH, Regan DG, Grulich aE, et al. Genital warts in young Australians five years into national human papillomavirus vaccination programme: national surveillance data. BMJ. 2013;346:2032–2032.

[21] Chow EPF, Read TRH, Wigan R, Donovan B, Chen MY, Bradshaw CS, et al. Ongoing decline in genital warts among young heterosexuals 7 years after the Australian human papillomavirus (HPV) vaccination programme. Sexually Transmitted Infections. 2015;91(3):214–219.

[22] DNA Genotek, Inc. Oragene-RNA (RE-100); 2017. Available from: http://www.dnagenotek.com/US/products/RE100.html.

[23] Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey (NHANES): HPV rinse; 2009.

Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2009-2010/manuals/HPV.pdf.

[24] DNA Genotek, Inc. OMNIgene-ORAL (OM-505); 2017. Available from: http://www.dnagenotek.com/US/products/OM505.

[25] Eve Medical. Eve Medical products: HerSwab; 2017. Available from: http://www.eve-medical.com/products.

[26] Gottschlich A, Rivera-Andrade A, Grajeda E, Alvarez C, Montano CM, Meza R. Acceptability of Human Papillomavirus SelfSampling for Cervical Cancer Screening in an Indigenous Community in Guatemala. Journal of Global Oncology. 2017;3(5):444– 454.

[27] Newman M. Networks: An Introduction. 2010. Oxford University Press.

[28] Petrie JG, Eisenberg MC, Ng S, Malosh RE, Han Lee K, Ohmit SE, et al. Application of an Individual-Based Transmission Hazard Model for Estimation of Influenza Vaccine Effectiveness in a Household Cohort. American journal of epidemiology;.

[29] Aiello AE, Simanek AM, Eisenberg MC, Walsh AR, Davis B, Volz E, et al. Design and methods of a social network isolation study for reducing respiratory infection transmission: The eX-FLU cluster randomized trial. Epidemics. 2016;15:38–55.

[30] Fan K, Eisenberg M, Walsh A, Aiello A, Heller K. Hierarchical graph-coupled hmms for heterogeneous personalized health data. In: Proceedings of the 21th ACM SIGKDD International Conference on Knowledge Discovery and Data Mining. ACM; 2015. p. 239–248.

[31] Kaplan EH, Heimer R. HIV prevalence among intravenous drug users: model-based estimates from New Haven's legal needle exchange. JAIDS Journal of Acquired Immune Deficiency Syndromes. 1992;5(2):163–169.

[32] Sloot PM, Ivanov SV, Boukhanovsky AV, van de Vijver DA, Boucher CA. Stochastic simulation of HIV population dynamics through complex network modelling. International Journal of Computer Mathematics. 2008;85(8):1175–1187.

[33] Cohen T, Colijn C, Finklea B, Murray M. Exogenous re-infection and the dynamics of tuberculosis epidemics: local effects in a network model of transmission. Journal of The Royal Society Interface. 2007;4(14):523–531.

[34] University of Michigan. Responsible Conduct of Research (RCR) Training; 2017. Available from: http://research-compliance. umich.edu/responsible-conduct-research-rcr-training.

[35] Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. The New England Journal of Medicine. 1998;338(7):423–8.

[36] Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, Désy M, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. Journal of Infectious Diseases. 1999 nov;180(5):1415–23.

[37] Molano M, Van den Brule A, Plummer M, Weiderpass E, Posso H, Arslan A, et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. American Journal of Epidemiology. 2003 sep;158(5):486–94.

[38] Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Thompson PJ, et al. Sequential acquisition of human papillomavirus (HPV) infection of the anus and cervix: the Hawaii HPV Cohort Study. The Journal of Infectious Diseases. 2010;201(9):1331–9.

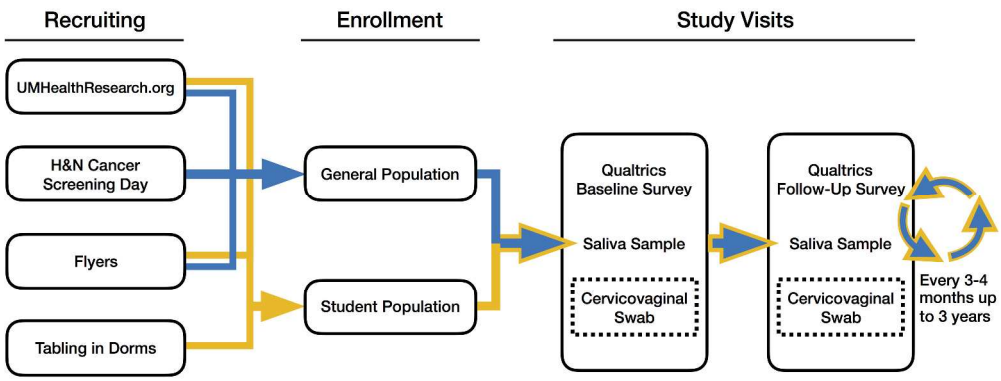

Figure 1: Flow diagram for the study detailing recruitment methods, visit structure (baseline and followups), and the cervicovaginal sub-study.

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Dynamics and Determinants of HPV Infection from the Michigan HPV and Oropharyngeal Cancer (M-HOC) Study: Study Protocol

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Dynamics and Determinants of HPV Infection from the Michigan HPV and Oropharyngeal Cancer (M-HOC) Study: Study Protocol

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Abstract

Introduction: Human papillomavirus (HPV) is the primary cause of cervical and other anogenital cancers and is also associated with head and neck cancers. Incidence of HPV-related oropharyngeal squamous cell cancers (OPSCCs) is increasing, and HPV-related OPSCCs have surpassed cervical cancer as the most common HPV-related cancer in the U.S. Given the multisite nature of HPV, there is strong interest in collecting data from both genital and oral sites, as well as

associated data on social and sexual behaviors. The overarching goal of this study is to evaluate patterns of oral HPV infection incidence, clearance, and persistence and their relationship to sexual behavior history.

Methods and Analysis: Participants are recruited from two populations: college students at a large public university and general population from the surrounding area. At the first study visit participants complete a detailed sexual history, health, and behavior questionnaire. Follow-up visits occur every 3–4 months over 3 years, when participants complete an abbreviated questionnaire. All participants provide a saliva sample at each visit, and eligible participants may provide a cervicovaginal self-swab. Genetic material isolated from specimens is tested for 15 high-risk and 3 low-risk HPV types. Statistical analyses will examine outcome variables including HPV prevalence, incidence, persistence, and clearance. Logistic regression models will be used to estimate odds ratios and 95% confidence intervals for associations between the outcomes of interest and demographic/behavioral variables collected in the questionnaires. The longitudinal HPV infection data and detailed sexual history data collected in the questionnaires will allow us to develop individual-based network models of HPV transmission and will be used to parameterize multi-scale models of HPV-related OPSCC carcinogenesis.

Ethics and Dissemination: This study has been approved by the University of Michigan Institutional Review Board. All participants are consented in person by trained study staff. Study results will be disseminated through peer-reviewed publications.

Article Summary

Strengths and Limitations of this study

- Longitudinal design with 3–4 study visits per participant per year allows for analysis of incidence, persistence, and clearance of oral HPV.

- Collection of both oral and cervicovaginal samples allows for comparison of infections across sites.
- Low prevalence of high-risk oral HPV may limit our ability to connect HPV infection dynamics to demographic, sexual, and behavioral variables.
- Participant-dependent saliva production and swishing effectiveness may result in variable sample quality, but this concern is mitigated by PCR sensitivity.
- Our HPV test separately identifies 15 high-risk genotypes but only 3 low-risk genotypes and thus may not provide a complete picture of the patterns of mucosal HPV types.

Introduction

Infectious disease agents account for approximately 20% of cancers worldwide [1]. The human papillomavirus (HPV) is the primary cause of cervical and most anogenital cancers, as well as an increasing fraction of head and neck cancers [2, 3]. Indeed, incidence of HPV-related oropharyngeal squamous cell cancers (OPSCCs) is increasing [4–7], and HPV-related OPSCCs have surpassed cervical cancer as the most common HPV-related cancer in the US [3]. At the University of Michigan Cancer Center, over 80% of OPSCC patients of have high-risk HPV detected in their cancers [8]. Although many HPV infections are benign, high-risk HPV infections can disrupt several key cancer regulation pathways, including particularly those involving tumor suppressors p53 and pRb [9, 10].

The underlying reasons behind the significant rise in HPV-related OPSCC rates in the U.S. are unclear. From a prevention perspective, addressing HPV-related OPSCC begins with an understanding of the epidemiology of the virus, both in the oral cavity and more broadly. Estimates from the National Health and Nutrition Examination survey (NHANES) suggest oral HPV prevalence in the U.S. is about 11–12% in men and 3–4 % in women [11–14]. However,

while oral HPV infections may clear relatively quickly (the HPV in Men trial estimated a mean clearance time of approximately 7 months [15]), persistent infections may lead to cancer after several decades (although there are no precise estimates of time-to-cancer for OPSCCs, cervical lesions are estimated to progress to cancer in 10–30 years [16]).

While studies are increasingly treating HPV as a multisite disease, the mechanisms underlying the relationship between oral, genital, and anal HPV infections are not understood, although autoinoculation is increasingly implicated [17, 18]. The HPV vaccine further complicates the epidemiological picture: HPV vaccination is expected to have an impact on the rates of oral HPV incidence, at least for those genotypes included in the vaccine [12, 19]. Countries with more aggressive vaccination campaigns, such as Australia, have already seen a reduction in HPV-related genital warts [20, 21]. It remains to be seen whether HPV vaccination will ultimately reduce the incidence and mortality of HPV-related head and neck cancers.

Many open questions remain regarding oral HPV transmission, epidemiology, infection and persistence, the mechanisms of HPV-related HN carcinogenesis, and the connection between the ongoing oral HPV epidemic and the rising OPSCC incidence. The knowledge gaps surrounding the mechanisms and epidemiology of HPV in the oral cavity hamper our ability to develop more effective measures to prevent oral HPV infections and, in turn, reduce the burden of head and neck cancers. Prevalence studies can only draw associations between the burden and probable risk factors. Thus, longitudinal studies with frequent testing and assessment of recent sexual behavior are needed to understand HPV transmission, persistence, and clearance and to associate these events with certain behaviors. Here, we describe the methods, study design, and initial study population characteristics of a longitudinal study of oral HPV incidence, clearance, and related sexual behaviors; this study and its cervicovaginal HPV sub-study are the epidemiological arms of the Michigan HPV and Oropharyngeal Cancer (M-HOC) Study. Our research will contribute to a better understanding of the complex factors affecting patterns of

HPV infection. This study will inform predictive and mechanistic models and develop effective prevention strategies for OPSCC and other HPV-related cancers.

Methods and Analysis

Objective

The overarching goal of this study is to evaluate patterns of oral HPV infection incidence and clearance and their relationship to sexual history and sexual behaviors. Participants are recruited to the study from two populations: college students at the University of Michigan (UM) (a large public university) and the general population of the surrounding area. As a secondary goal, we will also compare HPV infection patterns in the oral and cervicovaginal sites as part of a sub-study examining cervicovaginal HPV infection.

Subject enrollment and consent

Here, we describe the process for subject enrollment and consent (diagrammed in Figure 1).

Identification of Study Subjects. Participants are continuously recruited by one of three methods. 1) Advertisement: we place recruitment announcements in UM health clinics and area community sites (e.g. libraries, coffee shops, bus stops), as well as on UM clinical research volunteer's website (www.umclinicalstudies.org). In this method, individuals who are potentially interested in participating in the study contact the study coordinator to determine eligibility. 2) Screening clinics: we approach participants of the UM health system free throat cancer screening clinic to invite eligible participants to join the study. 3) Student residence halls: once

per year, we set up information tables in or near UM residence halls and invite eligible students to join the student.

Eligibility Criteria. Volunteers are considered for enrollment if they

- Are age 18 or older,
- Do not have a history of head or neck cancer,
- Are willing to return for a follow up every 3–4 months over 3 years,
- And are able and willing to provide written informed consent.

To participate in the optional cervicovaginal sub-study, participants must additionally

- Have a vagina,
- Not be pregnant,
- And not be menstruating at the time of the study visit.

Consent. We document informed consent for all willing and eligible participants before enrollment in the study. Separate, additional consent is obtained prior to enrollment in the cervicovaginal sub-study. All study subjects are given the opportunity to consent to storage of residual specimens collected in this study for future research purposes. Specimens collected from subjects who do not consent to specimen banking will be destroyed after completion of the analyses of this study.

Benefit to participants. Since we are not using a clinical test, our IRB approval does not allow returning individual HPV test results to participants, but population-level results will be disseminated through newsletters after peer-reviewed publication. Staff are knowledgeable and available to discuss vaccination and screening with interested participants. Because of the

nature of the sexual and behavioral questionnaire, we also have pamphlets for the UM Sexual Assault Prevention and Awareness Center available to participants.

Enrollment and statistical power. Phase I of this study recruited between April 2015 and December 2017, enrolling 395 participants. At this sample size and level of significance 0.05, we will detect the difference at baseline between two equally sized populations with HPV prevalence 10% and 20% with 80% power. Assuming each participant completes 10 visits, we will detect the difference between 10% and 13% ever HPV positive with more than 80% power. At the time of submission, 321 participants had completed at least one follow-up visit, and 1,693 baseline and follow-up visits had been completed. Follow-up visits are ongoing. Pending funding, phase II is anticipated to recruit a similar number of participants (potentially with additional study locations as well).

Figure 1: Flow diagram for the study detailing recruitment methods, visit structure (baseline and follow-ups), and the cervicovaginal sub-study.

Data collection

We administer surveys and collect biological specimens as described below.

Social and Sexual Behavior Survey. All study participants complete a comprehensive questionnaire at the baseline visit and an abbreviated questionnaire at all subsequent visits. The questionnaire consists of questions related to demographic information; behavior, including alcohol, cigarette, cigar, tobacco, and other drug use; and sexual practices, sexual history, sexual healthcare (including HPV vaccination status), general health, and history of sexually

transmitted infections (STIs). The baseline questionnaire collects a complete sexual history, with subsequent follow up visits providing more recent information and updates. The questions regarding sexual behaviors and history are designed to provide detailed information about the timing of different sexual partnerships and sexual practices in each sexual partnership and form a longitudinal, egocentric sexual network data set. Table 1 details the categories and numbers of questions given in both the baseline and follow-up surveys. The questionnaire is administered electronically via Qualtrics in a quiet, private room. Participants are asked to enter their unique study identification number at the beginning of the questionnaire. Participants are free to skip or otherwise not answer any question.

Table 1: Categories and numbers of questions asked in MHOC study baseline and follow-up questionnaire.

	Number of questions	
Question category	Baseline	Follow-up
Demographic	8	5
Oral health and cancer	5	5
STI testing and diagnosis	17	12
Sexual health and vaccinations	25	16
Sexual behavior	70	6
Sexual partners	22	18
Substance use	20	16

Oral Specimen Collection. Two mL of saliva and oral rinse specimen are collected at each visit (i.e. baseline and every 3-4 months for 3 years) using Oragene RE-100 kits [22]. Participants may use a sweetener or candy to stimulate saliva production if needed.

In the initial phase of the study, we tested several alternative saliva collection methods: Scope-brand mouthwash (administered as in NHANES [23]), and two commercially available kits developed for detection of DNA and RNA in saliva, the OM-505 (suitable for DNA, stable for weeks at room temperature) and RE-100 (designed to protect RNA, stable for 60 days at room

temperature) [22, 24]. We were able to detect DNA from saliva in all three methods but were only able to reliably recover RNA from the RE-100 kit sample. We selected the RE-100 kits for use in the study so that we could isolate both DNA and RNA from a single sample.

Cervicovaginal swab collection. Participants fulfilling the eligibility criteria are given the option at each study visit to provide a self-collected cervicovaginal swab sample using a HerSwab [25] in addition to the oral specimen. The HerSwab is a vaginal swab designed to sample near the cervix. Participants self-administer the swab by inserting the tip of the HerSwab into the vagina; turning the crank so that the brush is fully extended; removing the swab; turning the crank the opposite direction to retract the brush back into the tip; and placing the HerSwab into a provided resealable plastic bag. The participants are provided with the printed, graphical instructions designed by the manufacturer and collect their sample in a restroom. Staff facilitating the study are available to review the procedure and answer any participant questions.

Cervicovaginal swab acceptability survey. All study participants who provide a cervicovaginal sample complete a questionnaire after each cervicovaginal self-collection that asks about comfort, ease-of-use, and subjective assessment of the self-collection procedure [26]. The questionnaire is administered electronically via Qualtrics.

Follow-up visit schedule

Participants are followed for up to three years after their baseline visit, for a total of up to 12 study visits. The study prioritizes longitudinal follow-up over regular follow-up, but strives to achieve both. Therefore, if participants miss a follow-up visit, they are not excluded and instead continue the study when next available. This protocol facilitates research in the student population, as students are often gone for extended periods of time on scheduled breaks.

Sample processing and analysis

Sample processing and DNA extraction differs for oral and cervicovaginal samples, but DNA analysis is the same for both kinds of samples.

Oral sample processing. DNA isolation from saliva samples is performed by first splitting the sample in half, half for DNA and half for RNA. The saliva is heated to 50˚C for 60 minutes, 1/25th volume of prepIT-L2P (DNA Genotek) is added. Sample is incubated for 10 minutes at 4˚C and spun for 20 minutes at 4100 rpm. After the supernatant is added to equal volumes of 95% ethanol to precipitate the DNA. The DNA is pelleted, washed with 200 μL of 70% ethanol and dried. The DNA pellet is rehydrated with Tris-EDTA buffer and quantified with picogreen (Invitrogen P11496) or the QuBit (Invitrogen Q32850). RNA is isolated from samples that have tested positive for HPV DNA. RNA isolation from saliva samples is performed using the Oragene RNA isolation method according to the manufacturer's instruction which includes QIAgen's RNeasy Micro Kit (74004). In short, the samples are heated to 50˚C for 60 minutes, 95˚C for 15 minutes, 20 μL neutralization solution is added. Samples then are incubated on ice for 10 minutes followed by the RNeasy Kit protocol. The RNA is then converted to cDNA for further testing. The cDNA synthesis is preformed using Invitrogen's Superscript III protocol and reagents (18080-044).

Cervicovaginal sample processing. After collection, the HerSwab is soaked for one minute in 20 mL of ThinPrep solution and subsequently swished vigorously for one minute. The 20 mL solution is then split evenly into two 15 mL nuclease-free conical tubes—one for RNA processing and one for DNA—and the tube for RNA is centrifuged. The pellet for RNA use is resuspended in RNAlater for isolation and later study. DNA and RNA processing is the same as above. For RNA isolation, the cells in RNAlater are pelleted by centrifugation and RNA is isolated using the RNeasy as described above. Qiagen reagents are used for DNA isolation.

The cells in the ThinPrep sample are pelleted, resuspended in cell lysis buffer, incubated with Proteinase k, treated with protein precipitation buffer, vortexed vigorously, pelleted and placed on ice for 10 minutes. The supernatant is transferred to isopropanol in glycogen solution mixed, and the DNA pellet is washed twice with cold ethanol, re-hydrated in hydration solution and dissolved at 65°C, and DNA is quantified with picogreen or QuBit.

DNA analysis. DNA and cDNA samples are assayed using a previously described, highly-sensitive method [8]. Briefly, multiplex competitive PCR amplification of the heterogeneous E6 region of 15 discrete high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73) and 3 low-risk (6, 11, 90) HPV types is performed, followed by probe-specific single base extension. Extension products are loaded onto a matrix silicon chip array and separated by size using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectroscopy, allowing detection of any HPV types present in the sample.

Statistical analysis

Outcomes of interest include, but are not limited to, HPV prevalence (detection of HPV, or detection of a specific HPV genotype), incidence (detection of HPV in a previously uninfected person, or detection of a specific HPV genotype in a person who previously tested negative for that genotype), persistence (detection of HPV at subsequent study visits, or detection of specific genotypes at subsequent study visits), and clearance (non-detection of HPV in a previously infected person, or non-detection of a specific HPV genotype in a person previously infected by that genotype). Other patterns of HPV detection, such as patterns of intermittent detection of the same genotype or detection of an HPV genotype at the oral site after previous detection at the genital site (or vice versa), will be considered. We will adjust for the time between visits as appropriate. Presence of RNA in addition to DNA will be used to distinguish between active and latent infections.

Covariates of interest include, but are not limited to, sex, age, student status, HIV-status, sexual orientation, HPV vaccine status, circumcision status, number of vaginal, oral, and anal sexual partners, timing and nature of recent sexual activity, use of sexual protection, and substance use. Demographic and sexual behavior differences among subpopulations will be considered.

Logistic regression models will be used to estimate odds ratios and 95% confidence intervals for associations between the outcomes of interest and the demographic/behavioral variables. Variables that are significant in univariate analysis or are considered to be relevant based on a priori knowledge will be evaluated in multiple logistic regression models. Cox regression models will be used to investigate the effect of covariates on clearance. Analyses will be performed in SAS or R statistical software.

Modeling analysis

The longitudinal HPV infection data and detailed sexual partner and sexual history data collected in the questionnaires will allow us to develop individual-based network models of HPV transmission. While the data from the study are necessarily egocentric (i.e. we cannot link partners reported by participants to any specific individual in or out of the study to form a full sexual network), they can nonetheless be used to directly parameterize simulation models of transmission. The models we develop can match the timing of partner switching, numbers of partners, and type of sexual contact, as well as a range of behavioral and demographic covariates as well as HPV infections and clearance times. We will draw from the measured distributions and correlation patterns of these variables in our population to generate simulated populations with sexual behavior patterns similar to those measured in our study (e.g. using approaches based on configuration model methods [27] to connect our simulated sexual network based on partner history data). This will allow us to generalize our statistical model findings to different populations and to examine counterfactual scenarios of alternative HPV

vaccine coverage levels. Similar models have been developed for other studies, both for sexually transmitted infections and other infectious diseases [28–33]. This study is designed to specifically inform such modeling analyses.

Patient and Public Involvement

Patients and the public were not involved in the development of this study. Study results will be disseminated to the public through peer-reviewed publications as described in the ethics and dissemination section. Additionally, the newsletter described below will be made accessible to the public through the study website.

Ethics and dissemination

The MHOC study was approved by the University of Michigan Institutional Review Board on August 26, 2014 (IRB # HUM00090326). This study was deemed to not require additional safety monitoring beyond the monitoring of the institutional review board. All study staff interacting with participants and participant data complete the University of Michigan Program for Education and Evaluation in Responsible Research and Scholarship (PEERRS) prior to beginning the project [31]. Informed consent is obtained from each participant by the study coordinator and PEERRS certified research assistants. Study results will be disseminated through a series of peer-reviewed publications. Study participants will receive a study results newsletter written for a general audience.

Discussion

The knowledge gained from this study will contribute to the field of HPV research and will be disseminated via peer reviewed publications and presentations at national and international conferences. This study will provide improved estimates of HPV clearance rates in the oral cavity, due to the short follow-up times, and will also allow us to examine how HPV clearance is affected by a range of factors, including coinfection with multiple HPV types. More broadly, this study will improve our understanding of the relationships between behavior patterns and HPV, helping to elucidate how sexual behaviors, numbers of partners, and other behaviors (e.g. alcohol and substance use) relate to HPV infection patterns. Additionally, this study will provide detailed data on HPV transmission in the college student population—a key population who are also seeing changing vaccination patterns as HPV vaccine coverage improves. This will allow us to further examine the impact of HPV vaccination on the incidence and prevalence of a range of vaccine types (both those covered by the vaccine and others). The study questionnaire and longitudinal design also are also useful in developing sexual network models of transmission, which will allow us to examine alternative vaccination strategies and generalize to other populations.

Limitations and Strengths

Because oral HPV prevalence in the United States is relatively low (prevalence of high-risk oral HPV among adults 18–69 was 4.0% in 2011–2014: 6.8% in men and 1.2% in women [14]), there is a risk that number of HPV positive samples in the study will be low, limiting our abilities to draw inference between HPV infection and the demographic and behavioral variables of the participant. The quality of our saliva and oral rinse specimens may depend on the saliva production and swishing efficacy of each participant, although this is mitigated by the sensitivity

of the PCR analysis. Finally, we only test for 18 genotypes, which, although we cover all high-risk types, may not give as complete a picture of patterns of mucosal HPV infection.

Instead of identifying risk factors through associations in cross-sectional prevalence analyses, we will use a longitudinal approach with frequent oral and cervicovaginal HPV testing and updated detailed sexual behavior questionnaires. This will allow for understanding not only prevalence of HPV but also incidence, persistence, and clearance. Some studies have also adopted a longitudinal approach (e.g. [34–38]), but many have had long times between follow-up or have focused only on sex or only on genital infection sites. In the MHOC study, we use short follow-up times, with 3–4 visits per participant per year. We focus primarily on oral infection in both men and women but also collect cervicovaginal samples to assess multisite infections.

Author's contributions

The study was conceptualized and funding was obtained by MCE, TEC, and RM. Methodology and original protocols were developed by MCE, LPC, AFB, HMW, BMM, YKL, TBT, RLD, CMG, TEC, and RM. Project administration is managed by LPC and YKL. MCE, LPC, AFB, HMW, BMM, YKL, CG, TEC, and RM are responsible for supervision of study implementation, staff, and students. The original draft was prepared by MCE with the assistance of LPC, TSS, and MLY. Review and editing was completed by MCE, LPC, AFB, HMW, BMM, YKL, TEC, and RM.

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Competing interests statement

All authors declare no conflicts of interest.

References

[1] Bouvard V, Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, et al. A review of human carcinogens–Part B: biological agents. The Lancet Oncology. 2009;10(4):321–322.

[2] Gillison ML, Alemany L, Snijders PJF, Chaturvedi A, Steinberg BM, Schwartz S, et al. Human papillomavirus and diseases of the upper airway: head and neck cancer and respiratory papillomatosis. Vaccine. 2012 nov;30 Suppl 5:F34–54.

[3] Jemal A, Simard EP, Dorell C, Noone AM, Markowitz LE, Kohler B, et al. Annual Report to the Nation on the Status of Cancer,

1975-2009, Featuring the Burden and Trends in Human Papillomavirus (HPV)-Associated Cancers and HPV Vaccination Coverage Levels. Journal of the National Cancer Institute. 2013;105(3):175–201.

[4] Chaturvedi AK, Engels EA, Anderson WF, Gillison ML. Incidence trends for human papillomavirus-related and -unrelated oral squamous cell carcinomas in the United States. Journal of Clinical Oncology. 2008;26(4):612–9.

[5] Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. Journal of Clinical Oncology. 2011;29(32):4294–301.

[6] Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. Journal of Clinical Oncology. 2013 dec;31(36):4550–9.

[7] Chaturvedi AK, D'Souza G, Gillison ML, Katki HA. Burden of HPV-positive oropharynx cancers among ever and never smokers in the U.S. population. Oral Oncology. 2016;60:61–67.

[8] Walline HM, Komarck C, McHugh JB, Byrd SA, Spector ME, Hauff SJ, et al. High-Risk Human Papillomavirus Detection in Oropharyngeal, Nasopharyngeal, and Oral Cavity Cancers. JAMA Otolaryngology–Head & Neck Surgery. 2013;139(12):1320.

[9] Doorbar J, Quint W, Banks L, Bravo IG, Stoler M, Broker TR, et al. The biology and life-cycle of human papillomaviruses. Vaccine. 2012;30 Suppl 5:F55–70

[10] McLaughlin-Drubin ME, Munger K. Oncogenic activities of human papillomaviruses. Virus Research. 2009;143(2):195–208.¨

[11] Gillison ML, Broutian T, Pickard RKL, Tong Zy, Xiao W, Kahle L, et al. Prevalence of oral HPV infection in the United States, 2009-2010. Journal of the American Medical Association. 2012;307(7):693–703.

[12] Brouwer AF, Eisenberg MC, Carey TE, Meza R. Trends in HPV cervical and seroprevalence and associations between oral and genital infection and serum antibodies in NHANES 2003-2012. BMC Infectious Diseases. 2015;15(1):575.

[13] Sonawane K, Suk R, Chiao EY, Chhatwal J, Qiu P, Wilkin T, et al. Oral Human Papillomavirus Infection: Differences in Prevalence Between Sexes and Concordance With Genital Human Papillomavirus Infection, NHANES 2011 to 2014. Annals of Internal Medicine. 2017; 167(10):714-724.

[14] McQuillan G, Kruszon-moran D, Markowitz LE, Unger ER, Paulose-ram R. Prevalence of HPV in Adults Aged 18–69: United States, 2011–2014. NCHS Data Brief. 2017;280.

[15] Kreimer AR, Campbell, CMP, Lin H, Fulp W, Papenfuss MR, Abrahamsen M, Hildesheim A, Villa LL, Salmerón JJ, Lazcano-Ponce E, Giuliano AR. Incidence and clearance of oral human papillomavirus infection in men: the HIM cohort study. The Lancet. 2013;382(9895):877–887.

[16] McCredie M, Sharples KJ, Paul C, Baranyai J, Medley G, Jones RW, Skegg DCG. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. The Lancet Oncology. 2008;9(5):425–434.

[17] Pamnani SJ, Nyitray AG, Abrahamsen M, Rollison DE, Villa LL, Lazcano-Ponce E, et al. Sequential Acquisition of Anal Human Papillomavirus (HPV) Infection Following Genital Infection among Men Who Have Sex with Women: The HPV Infection in Men (HIM) Study. Journal of Infectious Diseases. 2016;214(8):1180–1187.

[18] Ranjeva S, Baskerville EB, Dukic V, Villa L, Lazcano-Ponce E, Giuliano AR, et al. Recurring infection with ecologically distinct human papillomavirus (HPV) types explains high prevalence and diversity. bioRxiv. 2017

[19] Markowitz LE, Liu G, Hariri S, Steinau M, Dunne EF, Unger ER. Prevalence of HPV After Introduction of the Vaccination Programin the United States. Pediatrics. 2016;137(3):1–9.

[20] Ali H, Donovan B, Wand H, Read TRH, Regan DG, Grulich aE, et al. Genital warts in young Australians five years into national human papillomavirus vaccination programme: national surveillance data. BMJ. 2013;346:2032–2032.

[21] Chow EPF, Read TRH, Wigan R, Donovan B, Chen MY, Bradshaw CS, et al. Ongoing decline in genital warts among young heterosexuals 7 years after the Australian human papillomavirus (HPV) vaccination programme. Sexually Transmitted Infections. 2015;91(3):214–219.

[22] DNA Genotek, Inc. Oragene-RNA (RE-100); 2017. Available from: http://www.dnagenotek.com/US/products/RE100.html.

[23] Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey (NHANES): HPV rinse; 2009.

Available from: https://wwwn.cdc.gov/nchs/data/nhanes/2009-2010/manuals/HPV.pdf.

[24] DNA Genotek, Inc. OMNIgene-ORAL (OM-505); 2017. Available from: http://www.dnagenotek.com/US/products/OM505.

[25] Eve Medical. Eve Medical products: HerSwab; 2017. Available from: http://www.eve-medical.com/products.

[26] Gottschlich A, Rivera-Andrade A, Grajeda E, Alvarez C, Montano CM, Meza R. Acceptability of Human Papillomavirus SelfSampling for Cervical Cancer Screening in an Indigenous Community in Guatemala. Journal of Global Oncology. 2017;3(5):444– 454.

[27] Newman M. Networks: An Introduction. 2010. Oxford University Press.

[28] Petrie JG, Eisenberg MC, Ng S, Malosh RE, Han Lee K, Ohmit SE, et al. Application of an Individual-Based Transmission Hazard Model for Estimation of Influenza Vaccine Effectiveness in a Household Cohort. American journal of epidemiology;.

[29] Aiello AE, Simanek AM, Eisenberg MC, Walsh AR, Davis B, Volz E, et al. Design and methods of a social network isolation study for reducing respiratory infection transmission: The eX-FLU cluster randomized trial. Epidemics. 2016;15:38–55.

[30] Fan K, Eisenberg M, Walsh A, Aiello A, Heller K. Hierarchical graph-coupled hmms for heterogeneous personalized health data. In: Proceedings of the 21th ACM SIGKDD International Conference on Knowledge Discovery and Data Mining. ACM; 2015. p. 239–248.

[31] Kaplan EH, Heimer R. HIV prevalence among intravenous drug users: model-based estimates from New Haven's legal needle exchange. JAIDS Journal of Acquired Immune Deficiency Syndromes. 1992;5(2):163–169.

[32] Sloot PM, Ivanov SV, Boukhanovsky AV, van de Vijver DA, Boucher CA. Stochastic simulation of HIV population dynamics through complex network modelling. International Journal of Computer Mathematics. 2008;85(8):1175–1187.

[33] Cohen T, Colijn C, Finklea B, Murray M. Exogenous re-infection and the dynamics of tuberculosis epidemics: local effects in a network model of transmission. Journal of The Royal Society Interface. 2007;4(14):523–531.

[34] University of Michigan. Responsible Conduct of Research (RCR) Training; 2017. Available from: http://research-compliance. umich.edu/responsible-conduct-research-rcr-training.

[35] Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. The New England Journal of Medicine. 1998;338(7):423–8.

[36] Franco EL, Villa LL, Sobrinho JP, Prado JM, Rousseau MC, Désy M, et al. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. Journal of Infectious Diseases. 1999 nov;180(5):1415–23.

[37] Molano M, Van den Brule A, Plummer M, Weiderpass E, Posso H, Arslan A, et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. American Journal of Epidemiology. 2003 sep;158(5):486–94.

[38] Goodman MT, Shvetsov YB, McDuffie K, Wilkens LR, Zhu X, Thompson PJ, et al. Sequential acquisition of human papillomavirus (HPV) infection of the anus and cervix: the Hawaii HPV Cohort Study. The Journal of Infectious Diseases. 2010;201(9):1331–9.

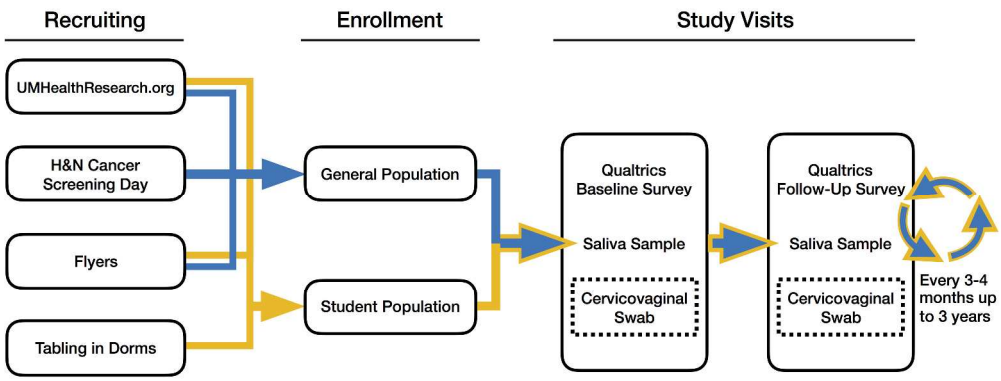

Figure 1: Flow diagram for the study detailing recruitment methods, visit structure (baseline and followups), and the cervicovaginal sub-study.

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